



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

April 29, 2019

SUBJECT: Review of Analytical Method Validation, Carbon and Nitrogen Transformation, and Environmental Fate Studies for Zinc Pyrithione for Studies Submitted by Thor GBMH to Support New Product Registration of ACTICIDE® ZP 100-F

FROM: James Breithaupt, Agronomist *James Breithaupt*
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

THRU: Laura Parsons, Acting Branch Chief *Laura Parsons*
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

TO: Eric Meiderhoff, Product Manager
Regulatory Management Branch I
Antimicrobials Division (7510P)

PC Code(s): 088002	DP Barcode(s)/No(s): D437466
Decision No.: 519871	Registration No(s): 67071-OA
Petition No(s): N/A	Regulatory Action: Review of Analytical Method Validation, Carbon and Nitrogen Transformation, and Environmental Fate Studies
Risk Assess Type: N/A	Case No(s): N/A
TXR No.: N/A	CAS No(s): 13463-41-7
MRID No(s): 497888-38, -42, -43, -44, -45, -47, -48, 49, -50, -51	40 CFR: N/A

INTRODUCTION:

To support the proposed new product registration of ACTICIDE® ZP 100-F, the registrant (Thor GBMH) has submitted ten studies on method validation, nitrogen and carbon transformation, and environmental fate of zinc pyrithione (PC Code 088002). Zinc pyrithione (PC Code 088002) is an active ingredient proposed to be used to control algae and bacteria in flooring and non-food contact adhesives, caulks, grouts, and sealants, residential and non-marine paints, latex emulsions, and drywall (Decision Number 519871). Use as an antifoulant paint is a primary pesticidal use for zinc pyrithione, but the antifouling use is not included in the June 20, 2018 label for this product¹.

¹ https://www3.epa.gov/pesticides/chem_search/ppls/067071-00101-20180620.pdf

BACKGROUND:

The studies in this memorandum were included in the February 24, 2017 data screen (D437931) which addressed the proposed use in dry film and/or in-can preservation of adhesives, building materials (including caulks, sealants, patching compounds, joint compounds, grouts, drywall, gypsum), polymers (including latex emulsions, thermoplastic resins), slurries (including mineral, pigment, and gum slurries), and architectural and industrial paints and coatings. These proposed uses of a new product, ACTICIDE® ZP 100-F, are consistent with the currently-registered uses.

DATA EVALUATION: The attached contains DERs for Activated sludge respiration inhibition (850.3300), Leaching-adsorption-desorption (835.1230), Hydrolysis (835.2120), Photodegradation in water (835.2240), Analytical method validation (850.6100), nitrogen and carbon transformation (OECD 216 and OECD 217), aerobic soil metabolism (835.4100), and WWTP simulation (Porous pot, 835.3220). The studies listed below were reviewed, and the results are located in Table 1 below.

CONCLUSIONS AND RECOMMENDATIONS:

The status of the data requirements is included in the table below.

Guideline	Guideline No.	MRID	DER #	Status
Hydrolysis	835.2120	49788848	1	Supplemental
Photodegradation in Water	835.2240	49788849	2	Supplemental
Aerobic soil metabolism	835.4100	49788845	3	Supplemental
Leaching-Adsorption-Desorption	835.1230	49788838	4	Acceptable
Activated Sludge Respiration Inhibition	850.3300	49788847	5	Acceptable
Porous Pot	835.3220	49788844	6	Acceptable
Analytical Method Validation (water)	850.6100	49788850	7	Acceptable
Analytical Method Validation (Soil)	850.6100	49788851	8	Acceptable
Soil Micro-Organisms: Nitrogen Transformation	OECD 216	49788842	9	Acceptable, not required
Soil Micro-Organisms: Carbon Transformation	OECD 217	49788843	9	Acceptable, not required

Data Evaluation Record 1


HYDROLYSIS OF ZINC PYRITHIONE (OCSPP 835.2120)


Report: Adam, D. (2015) Zn-[Carbon 14] Pyrithione: Hydrolysis at Three Different pH Values: Final Report. Project Number: 20130082. Unpublished study prepared by Innovative Environmental Services (IES) Ltd. 136p.

Document No.: MRID 49788848

Guideline: OCSPP 835.2120

PC Code: 088002

From: James Breithaupt, Agronomist 
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

Peer Review: Diana Hsieh, Biologist 
Risk Assessment and Science Support Branch (RASSB)
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EXECUTIVE SUMMARY

This study is classified as supplemental and satisfies the 835.2120 data requirement for ACTICIDE® ZP 100-F. Zinc pyrithione is expected to be essentially stable to hydrolysis at pH 4, 7, and 9 at ambient temperatures (25 °C). The half-lives calculated by the study author for temperatures between 25-60 °C ranged from 10 to 79 days, and the temperature-adjusted half-lives at 25 °C were 62-194 days. These temperature-adjusted half-lives were consistent with the half-lives of 99-123 days in another hydrolysis study.

This study (MRID 49788848) identified the only significant degradation product as pyridine sulfonic acid. Another hydrolysis study also identifies significant degradation products as pyrithione disulfide at pH 5 and 7 and pyridine sulfinic acid (pyridine sulfonic acid minus one oxygen)². This study is classified as supplemental given the fact that pyridine sulfonic acid is the terminal degradate observed in numerous environmental fate studies despite lack of quantification of intermediate degradation products.

² See Appendix A for chemical structures

I. MATERIALS AND METHODS

Guideline followed: OCSPP 835.2120

GLP Compliance: This study was conducted in compliance using Good Laboratories Practices (GLP) as published by the OECD Principles of Good Laboratory Practice (1999) and Swiss Ordinance Good Laboratory Practices, (2005). According to the registrant, these principles are consistent with those of U.S. EPA and Japan.

A. Materials:

1. Test Material: **ACTICIDE® ZP 100-F**, Zinc pyriothione (bis(2-pyridylthio)zinc 1,1'-dioxide)

IUPAC Name: Bis(2-pyridylthio)zinc 1,1'-dioxide

CAS Name: Bis(2-pyridylthio)zinc 1,1'-dioxide

CAS Number: **13463-41-7**

Molecular formula: C₁₀H₈N₂O₂S₂Zn

Molecular weight: 317.7

Description: Solid

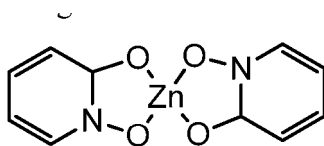
Batch Number: 4794NGP036-2

Purity: **ACTICIDE® ZP 100-F**, Radiopurity, 99.9 %, HPLC/UV purity, 97.55 %, Specific activity 5.02 MBq/mg;

Storage conditions: Ambient conditions.

SMILES String: c2n(cccc2)=O)S[Zn]Sc1n(cccc1)=O

Chemical Structure:



Buffer Solutions and Sterilization of Test System

Hydrolysis was measured at pH 4, 7, and 9 using citrate buffer (pH 4), phosphate buffer (pH 7), and borate buffer (pH 9). All buffer solutions were diluted to 1 liter using purified water and the final concentrations was 0.05 M. Buffer solutions and laboratory equipment were sterilized using an autoclave at 121 °C for 15 minutes.

Preliminary Test

The study used a preliminary test at 50 °C to determine if significant (≥ 10 %) hydrolytic degradation would occur. Solutions at pH 4, 7, and 9 containing 0.35-0.37 mg/L zinc pyrithione were studied at different sampling intervals up to 7 days for pH 4 and up to 60 days for pH 7 and 9. Table 1 below contains the pH, temperature, concentrations, and sampling intervals for both the preliminary test and the main test.

Main Test

A main test was conducted because ≥ 10 % degradation was observed in the preliminary study. Duplicate samples containing 0.31-0.36 mg/L zinc pyrithione were tested at pH 4, 7, and 9 for pH 4 (up to 30 days), pH 7 (30-60 days), and pH 9 (up to 60 days).

Table 1. Experimental Details for Preliminary and Main Tests

Test	pH	Temperature (°C)	Concentration (mg/L)	Sampling Intervals (days)
Preliminary	4	50	0.37	0, 1, 7
	7		0.35	0, 1, 7, 10, 18, 32
	9		0.35	0, 1, 1, 3, 7, 10, 18, 32
Main	4	25	0.34	0, 10, 30
		35	0.31	
	7	40	0.36	0, 7, 12, 18, 25, 30, 60
		60	0.36	0, 1, 7, 11, 15, 30
	9	25	0.31	0, 2, 3, 16, 20, 24, 30, 42, 60
		35	0.32	0, 3, 8, 13, 20, 30, 42, 60

Analytical Methods

HPLC and TLC were used in combination with UV detection (254 nm). For HPLC analysis, the limit of detection (LOD) was 2.3 $\mu\text{g/L}$ (0.7 %) and the limit of quantitation was 1.2 $\mu\text{g/L}$ (0.4 %). For LSC analysis, the limit of detection (LOD) was 2.0 $\mu\text{g/L}$ (5.9 %) and the limit of quantitation was 1.2 $\mu\text{g/L}$ (3.9 %).

II. Results and Discussion

Preliminary Study

In the preliminary study at 50°C, significant (≥ 10 %) of applied zinc pyrithione degraded at pH 4, 7, and 9. However, calculating a half-life at pH 4 was not possible because zinc pyrithione and its degradation products pyridine sulfonic acid (M1) and an unknown degradate (M3) reached equilibrium over the 7-day period (Table 4 of study). The half-life at pH 7 and 50 °C was 14 days with a temperature-adjusted half-life of 78 days for 25 °C, and the half-life at pH 9 and 50 °C was 11 days with a temperature-adjusted half-life of 62 days for 25 °C based on a

multiplication factor of $5.66^{3,4}$. The adjustment of the half-life from a higher temperature to 25 °C by temperature is based on the assumption that the rate of reaction declines with every 10 °C decrease in temperature with a corresponding increase in the half-life.

At pH 7, parent zinc pyriithione declined from 97 % of applied at day zero to 18 % at 32 days. The degradate pyridine sulfonic acid (M1) increased from 3 % at day zero to 86 % at day 32 (Table 5 of study). At pH 9, parent zinc pyriithione declined from 96 % at day zero to 24 % at day 32. The degradate pyridine sulfonic acid (M1) increased from 4 % at day zero to 76 % at day 32. No other degradation products were formed at ≥ 10 % of applied concentrations at pH 7 and 9 (Table 6 of study). In all the tested pH values, the material balance was within 90-110 % with the exception of the 10-day interval for pH 7 (87%) [Tables 4, 5, and 6 of study]. Table 2 contains the half-lives from both the preliminary and main study.

Main Study

The main study measured the degradation of zinc pyriithione at pH 4, 7, and 9 and was conducted because ≥ 10 % of applied parent compound degraded in the preliminary study at 50 °C. However, calculating a half-life at pH 4 was not possible because for both 25 °C and 35 °C, zinc pyriithione reached equilibrium with its pyridine sulfonic acid degradate (M1). Zinc pyriithione ranged from 72-81 % of applied at 25 °C and 78-84 % at 35 °C, and pyridine sulfonic acid ranged from 12-17 % at 25 °C and 8-17 % at 35 °C (Tables 7 and 8 of study).

pH 7

At 40 °C, parent zinc pyriithione declined from 100 % of applied at day zero to 18 % at 60 days. The degradate pyridine sulfonic acid (M1) increased from 11.9 % at day 7 to 43 % at day 60 (Table 9 of study). At 60 °C, parent zinc pyriithione declined from 100 % of applied at day zero to 7 % at 30 days. The degradate pyridine sulfonic acid (M1) increased from 11.2 % at day 7 to 96 % at day 60 (Table 10 of study). The half-life at pH 7 and 40 °C was 69 days with a temperature-adjusted half-life of 194 days for 25 °C, and the half-life at pH 7 and 60 °C was 10 days with a temperature-adjusted half-life of 110 days for 25 °C.

pH 9

At 25 °C, parent zinc pyriithione declined from 100 % of applied at day zero to 55 % at 60 days. The degradate pyridine sulfonic acid (M1) increased from 3.6 % at day 2 to 52 % at day 60 (Table 11 of study). At 35 °C, parent zinc pyriithione declined from 98 % of applied at day zero to 45 % at 60 days. The degradate pyridine sulfonic acid (M1) increased from 1.7 % at day 0 to 57 % at day 60 (Table 11 of study). The half-life at pH 9 and 25 °C was 79 days, and the half-life at pH 9 and 35 °C was 35 days with a temperature-adjusted half-life of 84 days for 25 °C (Table 12 of study).

³ $2^{(50-25)/10}=5.66$

⁴ Other temperature adjustment factors are presented in Table 2 below

Table 2. Half-lives in Preliminary and Main Tests

Test	pH	Temperature (°C)	Half-lives (days)	Temperature-adjusted half-life (25 °C) ^{1, 2}	Temperature Adjustment Factor	Comments
Preliminary	4	50	Not calculated	Not calculated	5.66 ³	Zinc pyriithione and pyridine sulfonic acid degradate reached equilibrium
	7		14	78		None
	9		11	62		
Main	4	25	Not calculated	Not calculated	1 ⁴	Zinc pyriithione and pyridine sulfonic acid degradate reached equilibrium
		35			2 ⁵	
	7	40	69	194	2.83 ⁶	None
		60			11.3 ⁷	
	9	25	79	Not applicable	1 ⁴	No temperature adjustment necessary
		35				
			42	84	2 ⁵	None

¹ Temperature Adjustment factor= $2^{((\text{Study temperature}-\text{reference temperature})/10)}$

² Multiply half-life by adjustment factor and divide rate constant by adjustment factor

³ $=2^{((50-25)/10)}=5.66$

⁴ Not adjusted because of no temperature difference

⁵ $=2^{((35-25)/10)}=2$

⁶ $=2^{((40-25)/10)}=2.83$

⁷ $(=2^{((60-25)/10)}=11.31$

III. STUDY DEFICIENCIES AND REVIEWER'S COMMENTS

The identification of the pyridine sulfonic acid degradation product is questionable given the fact that the identified degradation product in another hydrolysis study was pyriithione disulfide (dimer of pyriithione molecules) at pH 5 and 7 and pyridine sulfinic acid at pH 9. Pyridine sulfinic acid contains one less oxygen than pyridine sulfonic acid. However, pyridine sulfonic acid was also formed in photodegradation in water, anaerobic aquatic metabolism, and aerobic aquatic metabolism studies.

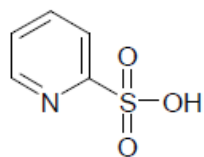
The presence of the degradate pyridine sulfonic acid is also questionable in MRID 49788848 because the sterility was demonstrated for all treatments except pH 7 and 40 °C (Table 3 of study).

IV. REFERENCES

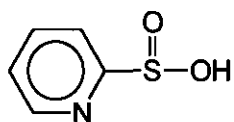
OECD 111. May 12, 1981. Hydrolysis as a Function of pH.
<http://www.oecd.org/chemicalsafety/risk-assessment/1948201.pdf>

U.S. EPA. November, 2008. OPPTS 835.2120: Hydrolysis. EPA 712-C-08-012.
<https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0152-0009>

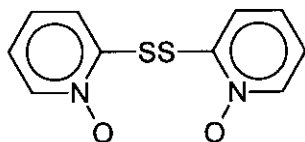
Appendix A. Zinc Pyrithione Degradation Products



Pyridine-2-sulfonic acid (M1)



Pyridine-2-sulfinic acid



Pyrithione disulfide

Data Evaluation Record 2


PHOTODEGRADATION IN WATER OF ZINC PYRITHIONE (OCSPP 835.2240)


Report: Adam, D. (2015) Zn-[Carbon 14] Pyrithione: Aqueous Photolysis in Buffer Solution and Natural Water: Final Report. Project Number: 20130084. Unpublished study prepared by Innovative Environmental Services (IES) Ltd. 100p.

Document No.: MRID 49788849

Guideline: OCSPP 835.2240

PC Code: 088002

From: James Breithaupt, Agronomist 
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Antimicrobial Division (7510P)

Peer Review: Diana Hsieh, Biologist 
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

EXECUTIVE SUMMARY

This study is classified as supplemental and satisfies the 835.2240 data requirement for ACTICIDE® ZP 100-F. In the pH 7 buffer solution, zinc pyrithione was essentially stable in the dark controls but degraded instantly in the irradiated treatment. In the pH 7 dark control, zinc pyrithione was essentially stable to hydrolysis with 6-13 % degradation overall by 17 days. In the natural water (pH 8.2-8.8), zinc pyrithione degraded instantly in both the irradiated and dark control treatments. In both the pH 7 buffer and natural water, significant (>10 % of applied) degradation products were formed but only pyridine sulfonic acid was identified. The study results for the dark control pH 7 buffer are consistent with the results of a hydrolysis study (MRID 43864602) where 10-14 % of parent zinc pyrithione degraded by 14-21 days.

I. MATERIALS AND METHODS

Guideline followed: OCSPP 835.2240

GLP Compliance: This study was conducted in compliance using Good Laboratories Practices (GLP) as published by the OECD Principles of Good Laboratory Practice (1999) and Swiss Ordinance Good Laboratory Practices, (2005). According to the registrant, these principles are consistent with those of U.S. EPA and Japan.

A. Materials:

1. Test Material: ACTICIDE® ZP 100-F, Zinc pyriithione (bis(2-pyridylthio)zinc 1,1'-dioxide)

IUPAC Name: Bis(2-pyridylthio)zinc 1,1'-dioxide

CAS Name: Bis(2-pyridylthio)zinc 1,1'-dioxide

CAS Number: 13463-41-7

Molecular formula: C₁₀H₈N₂O₂S₂Zn

Molecular weight: 319.09

Description: Solid

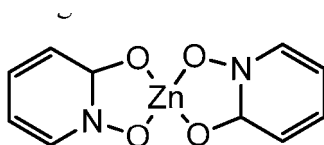
Batch Number: 4794NGP036-2

Purity: ACTICIDE® ZP 100-F, Radiopurity, 99.9 %, HPLC/UV purity, 97.55 %, Specific activity 5.02 MBq/mg;

Storage conditions: Ambient conditions.

SMILES String: c2(n(cccc2)=O)S[Zn]Sc1n(cccc1)=O

Chemical Structure:



Test Medium and Sterilization of Test System

Buffer solution (pH 7) with a final concentration of 0.05 M (mol/L) was prepared by diluting 296 mL of 0.1 N sodium hydroxide solution with 500 mL 0.1 M monopotassium phosphate and adding bi-distilled water to reach a final volume of one liter. The buffer solution was then sterilized before use by autoclaving at 121 °C for 15 minutes. Natural pond water (pH 8.2-8.8) was sampled from Biederthal, France, passed through a 0.2 mm mesh, and sterilized using gamma irradiation (γ -irradiation) at up to 40 kGy (kilograys). A kilogray is one joule of energy per kilogram of matter (Wikipedia). The laboratory equipment was also sterilized before use by autoclaving at 121 °C for 15 minutes.

Analytical methods

Liquid scintillation counting (LSC) was used to measure the quantity of radioactivity, and HPLC coupled with a UV detector (254 nm) was used to quantify the fractions in the samples. Thin layer chromatography (TLC) was used to confirm the HPLC results. For LSC analysis, the limit of detection (LOD) was 0.5 % and the limit of quantitation (LOQ) was 0.7 %. For HPLC analysis, the LOD was 0.3 % and the LOQ was 0.7 % (Table, p. 28 of study).

Main Test

Duplicate 100 mL samples of sterile buffer solution at pH 7 and natural pond water treated with the radiolabeled test item at a concentration of 0.29 mg/L (below half of the water solubility of the test item) were irradiated for a continuous period of 17 days. This continuous irradiation time corresponded to 32 days of midsummer sunlight at latitudes 30 to 50°N (includes 40 °N, U.S. EPA, 1993)⁵. Concurrently, 100 mL samples of the treated test solutions were incubated under the same conditions but in darkness. The samples were maintained at a mean temperature of 25.1 ± 0.2 °C (irradiated) and 25.9 ± 0.3 °C (dark control) throughout the study.

Volatility traps consisting of ethylene glycol and sodium hydroxide solutions were used to determine the formation of organic volatile compounds and radioactive carbon dioxide, respectively. Aliquots were taken from the test solutions after 0, 1, 3, 7, 10 and 17 days of irradiation and directly submitted to radiochemical quantification by Liquid scintillation counting and chromatographic analysis by High-performance liquid chromatography. The analytical results were confirmed by Thin-layer chromatography on selected samples.

II. Results and Discussion

pH 7 Buffer

There was minimal change in pH within the study duration for both the irradiated treatment (7.05-7.18) and the dark control treatment (7.0-7.1), and the test solutions were determined to be sterile. The material balances were 99-103 % of applied in the irradiated treatment and 106 % for the dark control treatment, and these values were within the acceptable range of 90-110 % for radiolabeled compounds. In the irradiated treatment, radioactivity in solution declined from 100 % at day zero to 86 % at day 1, and ¹⁴CO₂ increased to 15 % by day 17. Radioactivity in the organic trap (ethylene glycol) was < 0.1 %. However, in the dark control, radioactivity was only detected in the solution and not in the CO₂ or ethylene glycol traps (Tables 3 and 4 of study).

The half-life of parent zinc pyriithione in the irradiated treatment was <1 day with no detectable (≥ 0.3 %) quantities after day zero, and the registrant stated that zinc pyriithione “degraded instantly”. The only identified degradation product was pyridine sulfonic acid (M1), which was present only at <1 % on day zero. There were three unidentified significant (>10 %) of applied degradation products including M2, M4, and M8. M2 increased to 10-10.2 % of applied by 10-17 days, and M8 increased to 12-14 % by 1-17 days. M4 increased to 72 % at one day, and declined to 62-63 % by 7-17 days (Table 7 of study). In the pH 7 dark control, zinc pyriithione was essentially stable to hydrolysis with 6-13 % degradation overall by 17 days, which is consistent with the results of an hydrolysis study where 10-14 % of parent zinc pyriithione degraded by 14-21 days (MRID 43864602)⁶. M8 reached 12-13 % by 7-17 days, and was the only significant degradation product formed (Table 9 of study).

⁵ The use of 40 °N latitude was recommended in the “Pesticide Reregistration Rejection Rate Analysis - Environmental Fate.pdf” document dated 1993

⁶ Based on Table 8 of study

Natural Pond Water (pH 8.2-8.8)

The pH was more variable in the natural pond water because a pH buffer was not used. The pH ranged from 8.2-8.8 for both irradiated and dark-control samples, and the test solutions were determined to be sterile. The material balances were 96-101 % of applied in the irradiated treatment and 100-103 % for the dark control treatment, and these values were within the acceptable range of 90-110 % for radiolabeled compounds. In the irradiated treatment, radioactivity in solution declined from 100 % at day zero to 82 % at day 17, and $^{14}\text{CO}_2$ increased to 14 % by day 17. Radioactivity in the organic trap (ethylene glycol) was < 0.1 %. However, in the dark control, radioactivity was only detected in the solution and not in the CO_2 or ethylene glycol traps (Tables 5 and 6 of study).

The half-life of parent zinc pyriethione in the irradiated treatment was <1 day with no detectable (≥ 0.3 %) quantities after day zero, and the registrant stated that zinc pyriethione “degraded instantly.” There were three significant degradation products, including pyridine sulfonic acid (M1) and two unidentified degradates (M2 and M4). Pyridine sulfonic acid (M1) ranged from 7-16 % of applied from 10-17 days. M2 increased from 5.9 % at day 1 to 10 % at day 3, and declined to 7 % by day 17. M4 declined from 98 % at day 0 to 62.8 % at day 1 (Table 11 of study). In the dark control, zinc pyriethione was not detected (>0.3 %) after 0 days. M4 reached 98-103 % by 0-17 days, and was the only significant degradation product formed (Table 13 of study).

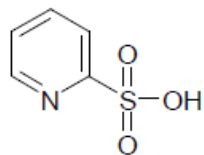
III. STUDY DEFICIENCIES AND REVIEWER’S COMMENTS

The identification of pyridine sulfonic acid degradation product is consistent with the fact that pyridine sulfonic acid is the terminal degradation product of zinc pyriethione in the environment. However, the primary degradation product in the irradiated portion of another photodegradation in water study was pyridine sulfonic acid, along with pyriethione disulfide (dimer of pyriethione molecules) and mixed disulfides at pH 9.

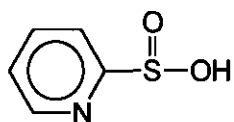
IV. REFERENCES

- MRID 43864602. Reynolds, J. (1995) Hydrolysis of (Pyridine-2,6-(carbon 14)) Zinc Omadine: Lab Project Number: XBL 94052: RPT00217: 94052. Unpublished study prepared by XenoBiotic Labs, Inc. 128 p.
- U.S. EPA. 1993. Pesticide Reregistration Rejection Rate Analysis - Environmental Fate.pdf.
- U.S. EPA. November, 2008. OPPTS 835.2240: Photodegradation in Water. EPA 712-C-08-013. <https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0152-0012>
- Wikipedia. Accessed March 15, 2013. [https://en.wikipedia.org/wiki/Gray_\(unit\)](https://en.wikipedia.org/wiki/Gray_(unit))

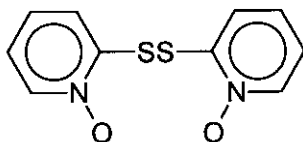
Appendix A. Zinc Pyrithione Degradation Products



Pyridine-2-sulfonic acid (M1)



Pyridine-2-sulfinic acid



Pyrithione disulfide

Data Evaluation Record 3


AEROBIC SOIL METABOLISM OF ZINC PYRITHIONE


Report: Volkel, W. (2015) Zn-[Carbon 14]Pyrithione - Route and Rate of Degradation in Four Soils Incubated Under Aerobic Conditions: Final Report. Project Number: 20130088. Unpublished study prepared by Innovative Environmental Services (IES) Ltd. 145p.

Document No.: MRID 49788845

Guideline: OCSPP 835.4100

PC Code: 088002

From: James Breithaupt, Agronomist 
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Peer Review: Diana Hsieh, Biologist 
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

EXECUTIVE SUMMARY

This study is classified as supplemental data and satisfies the 835.4100 data requirement for ACTICIDE® ZP 100-F. In four tested soils, zinc pyrithione degraded with half-lives of <30 minutes and formed six significant degradation products. The only identified degradation product was pyridine sulfonic acid, which is the terminal degradation product prior to mineralization.

I. MATERIALS AND METHODS

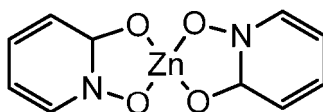
Guideline followed: 835.4100

GLP Compliance: This study was conducted in compliance with OECD Principles of Good Laboratory Practice (April, 1999), Directive 2004/10/EC of the European Parliament and of the Council of 11th February 2004, Part two, and OECD principles of Good Laboratory Practice, adopted by Council on 26th November 1997; Environment Directorate, Organisation for Economic Cooperation and Development, April 1999.

A. Materials:

1. Test Material: ACTICIDE® ZP 100-F, Zinc pyrithione (bis(2-pyridylthio)zinc 1,1'-dioxide)

CAS and IUPAC Name: Bis(2-pyridylthio)zinc 1,1'-dioxide
CAS Number: 13463-41-7
Molecular formula: C₁₀H₈N₂O₂S₂Zn
Molecular weight: 319.09 g/mol
Description: Solid
Batch Number: 4794NGP036-2 (no stated expiration date)
Purity: ACTICIDE® ZP 100-F, 99.9 %; 5.02 MBq/mg
Storage conditions: Ambient conditions.
SMILES String: c2(n(cccc2)=O)S[Zn]Sc1n(cccc1)=O
Chemical Structure:



This study used four (4) soils from Europe, 3 from Germany and 1 from Switzerland, and the chemical and physical properties may be seen in Table 1 of this DER and Table 2 of the study. These soils were collected from the 0-20 cm depth (0-8 inches) and sieved through a 2 mm sieve.

For each soil, 100 grams of soil were incubated (20.8 ± 0.3 °C) under aerobic conditions in glass metabolism flasks in darkness. The flasks were maintained at a field capacity⁷ of 10 kPa (kilopascals), which corresponds to 0.1 bar, or 75 mm Hg. The flasks were equipped with an air inlet and an air outlet with a KOH trap to catch CO₂ and an ethylene glycol trap to catch organic volatiles.

Each soil was treated with a nominal concentration of 0.5 mg/kg zinc pyriithione and taken for extraction and analysis at 0 and 6 hours, 1, 2, 7, 28, and 119 days. After the appropriate sampling interval, radioactivity in volatility traps was monitored using LSC, and CO₂ was precipitated using Ba(OH)₂.

Replicate soil samples were extracted using a Soxhlet flask with acetonitrile water for at least 4 hours and 0.01 M KOH solution. Reverse-phase HPLC⁸ was used to quantify zinc pyriithione and its degradation products in application solutions, supernatants, and soil extracts.

Table 1. Aerobic Metabolism in Four (4) Representative Soils¹.

Soil (Soil #)	Country of Origin	Texture	pH	% OC	% clay	CEC
Speyer 2.3 (I)	Germany	Sandy loam	6.9	0.97	8.9	9.1
Speyer 2.4 (II)		Loam	7.2	2.3	25.9	31.4
Am Fischteith		Silt loam	6.2	1.8	20.5	19.5

⁷ Field capacity is the amount of water a soil can hold against the force of gravity

⁸ Reverse-phase HPLC employs a polar mobile phase (liquid) and a hydrophobic column

Soil (Soil #)	Country of Origin	Texture	pH	% OC	% clay	CEC
(V)						
Witterswil (IV)	Switzerland	Silty clay loam	7.24	3.93	37.8	59.7

¹ Based on Table 2 of study, p. 37

II. Results and Discussion

Material Balance

In the four soils, Soxhlet extraction using acetonitrile:water (4:1) did not remove significant (≥ 10 % of applied) zinc pyriothione residues. Acetonitrile:water extractions did not contain more than 8.6 % of applied residue. However, the room-temperature extractions using NaOH did remove significant radioactivity from the treated soil. Extractable residues were initially 82-92 % of applied, and declined to 2.9-33 % of applied by 119 days (end of study). Non-extractable residues were initially 3-13 % of applied and increased to 10-28 % of applied by 119 days. Mineralization was the predominant route of dissipation with CO₂ reaching 52-65 % of applied by 119 days, but organic volatiles were not detected (>0.1 %) in the ethylene glycol traps. Overall, the material balance was 96-99 % of applied in all four soils.

In all 4 soils, the half-life of parent zinc pyriothione was <30 minutes. Even though numerous degradation products were present at ≥ 10 % of applied concentrations, the only identified degradation product was M2, or pyridine sulfonic acid, which was formed in significant quantities in all four soils (Tables 7-10 of study). Pyridine sulfonic acid ranged from 0.5-39 % of applied in soil with most concentrations being above 10 %, and it was generally detected later on in the study. This detection at later intervals is consistent with pyridine sulfonic acid being a terminal degradate prior to mineralization. Other unidentified degradation products present in the study at ≥ 10 % of applied included M1, M3, M4, M6, and M7 (See Table 2 of DER). Table 3 of this DER contains the half-lives of the degradation products M1 and M7 from the study, which ranged from 29-174 days for M1 and 0.1-14 days for M7.

Table 2. Formation Rate of Degradation Products of Zinc Pyriothione in Aerobic Soil.

Soil (Soil #)	Texture	% of Applied (min-max)					
		M1	M2 ¹	M3	M4	M6	M7
Speyer 2.3 (I) ²	Sandy loam	0.8-66	10-39	3.4-12	1.5-11	2.9-20	2.8-19
Speyer 2.4 (II) ³	Loam	2.8-44	0.5-19	0.6-13	0.5-11	0.6-20	14-34
Am Fischteith (V) ⁴	Silt loam	4.9-20	2-13	6-14	5.6-17	10-21	5-44
Witterswil (IV) ⁵	Silty clay loam	6-55	9-25	6-8	3.4-15	2.2-14	21-37

¹ M2 or pyridine sulfonic acid was the only identified degradation product

² Table 7, p. 42

² Table 8, p. 43

³ Table 9, p. 44

⁴ Table 10, p. 45

Table 3. First-Order Half-lives (Days) of M1 and M7 Metabolites in Four Aerobic Soils¹.

Soil (Soil #)	Texture	Half-life (days, r ²)	
		M1	M7
Speyer 2.3 (I)	Sandy loam	36 (0.85)	0.1 (0.57)
Speyer 2.4 (II)	Loam	48 (0.86)	3 (0.61)
Am Fischteith (V)	Silt loam	174 (0.74)	14 (0.88)
Witterswil (IV)	Silty clay loam	29 (0.99)	1.7 (0.94)

¹ Table 2 of study, p. 37

III. REFERENCES

U.S. EPA, Fate, Transport, and Transformation Test Guidelines. October, 2008. OPPTS 835.4100 Aerobic Soil Metabolism, OPPTS 835.4200 Anaerobic Soil Metabolism. [EPA 712-C-08-016], [EPA 712-C-08-017] <https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0152-0038>

Data Evaluation Record 4

SORPTION OF ZINC PYRITHIONE IN REPRESENTATIVE SOILS

Report: Volkel, W. (2015) Zn-[Carbon 14]Pyrithione- Adsorption/Desorption in Five Soils. Project Number: 20130086. Unpublished study prepared by Innovative Environmental Services (IES) Ltd. 123p.

Document No.: MRID 49788838

Guideline: OECD 106/OCSP 835.1230

PC Code: 088002

From: James Breithaupt, Agronomist *James Breithaupt*
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

Peer Review: Diana Hsieh, Biologist *Diana Hsieh*
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

EXECUTIVE SUMMARY

This study is classified as acceptable and satisfies the 835.1230 data requirement for ACTICIDE® ZP 100-F. This study includes adsorption experiments conducted with parent zinc pyrithione in five soils sterilized using gamma irradiation which does not cause damage to soil structure. The Freundlich Kads values ranged from 28-187 L/kg and the Koc values ranged from 2,088-8,293 L/kg. The fit of the Freundlich equation was good with r^2 values of 0.97-0.99 (Table 1 below). Sorption was most closely related to the organic carbon content of the soils ($r^2=0.85$) and the cation exchange capacity ($r^2=0.69$), which represents the combination of clay, organic carbon content and pH. Based on the Koc values in tested soils, parent zinc pyrithione is slightly mobile in soil.

I. MATERIALS AND METHODS

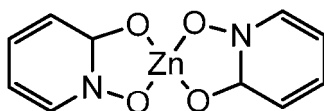
Guideline followed: 835.1230

GLP Compliance: This study was conducted in compliance with OECD Principles of Good Laboratory Practice (April, 1999), Directive 2004/10/EC of the European Parliament and of the Council of 11th February 2004, Part two, and OECD principles of Good Laboratory Practice, adopted by Council on 26th November 1997; Environment Directorate, Organisation for Economic Cooperation and Development, April 1999.

A. Materials:

1. Test Material: ACTICIDE® ZP 100-F, Zinc pyrithione (bis(2-pyridylthio)zinc 1,1'-dioxide)

CAS and IUPAC Name:	Bis(2-pyridylthio)zinc 1,1'-dioxide
CAS Number:	13463-41-7
Molecular formula:	C ₁₀ H ₈ N ₂ O ₂ S ₂ Zn
Molecular weight:	317.7 g/mol
Description:	Solid
Batch Number:	4794NGP036-2 (no stated expiration date)
Purity:	ACTICIDE® ZP 100-F, 99.9 %; 5.02 MBq/mg
Storage conditions:	Ambient conditions.
SMILES String:	c2(n(cccc2)=O)S[Zn]Sc1n(cccc1)=O
Chemical Structure:	



All five soils used in the study were from Germany and the chemical and physical characteristics may be seen in Table 1 of this DER and Table 1 of the study. The soils in this experiment ranged from loamy sand to clay textures, and were collected to a depth of 20 cm (8 inches), air-dried and ground to <2 mm particle size.

A preliminary screening test was performed using duplicate samples of two soils (Speyer 2.3 and 2.4) at a nominal concentration of 0.5 mg/L at soil:solution ratios of 1:25, 1:5, and 1:1. The preliminary test was conducted to determine the optimal soil-to-solution ratio, the stability of the test substance in control samples, and the extent of sorption to the test substance to test vessels. The test item concentrations were determined in the aqueous phase and the amount adsorbed to soil particles was calculated by difference to the initial amount of test item applied (indirect method). The results of these screening tests are reported in Table 2 of this DER.

Screening tests were performed with all five soils at one test item concentration (0.5 mg/L nominal) and for one defined soil-to-solution ratio. Based on the results the following three screening tests were performed.

Screening test I was performed using all five soils (non-sterilized) at a soil-to-solution ratio of 1:25 and duplicate samples in order to determine the adsorption kinetics at a single concentration and the distribution coefficients K_d and K_{oc} , the optimal equilibration time for adsorption and the amount of test item adsorbed at equilibrium.

Screening test II was performed using three sterilized soils (soil I, III and IV) at a soil-to-solution ratio of 1:25 and single samples in order to determine the adsorption kinetics at a single concentration and the distribution coefficients K_d and K_{oc} for sterilized soils to determine the influence of microbial degradation and the optimal equilibration time for adsorption and the amount of test item adsorbed at equilibrium for soils sterilized using gamma irradiation.

Screening test III was performed using all five soils sterilized at a soil-to-solution ratio of 1:50 and duplicate samples in order to determine the desorption kinetics at a single concentration and the distribution coefficients K_d and K_{oc} after adsorption/desorption, the optimal equilibration time for desorption and the amount of test item adsorbed/desorbed at equilibrium, and the mass balance for all soils at adsorption and desorption equilibrium.

In the Advanced Test, the sieved soils were put into Pyrex glass tubes with a ratio of 1gram soil and 45 ml of 0.01 M CaCl_2 (1:50 ratio) and shaken for 24 hours. Based on Screening test III, 24 hours was chosen as the equilibrium time in the final study. The test concentrations were 0.0022, 0.0058, 0.0181, 0.0634, and 0.2038 mg/l. After centrifuging for one (1) hour, the supernatants were removed and analyzed for zinc pyriithione and pH. Following the adsorption phase of the study, the supernatant was decanted and untreated 0.01 M CaCl_2 was then added to the tubes. After 24 hours of shaking, the desorption supernatants were decanted and the analyzed for zinc pyriithione and pH.

Table 1. Chemical and Physical Properties of Tested Soils¹.

Soil (Soil #)	Texture	pH	% OC	% clay	CEC
Speyer 2.3 (I)	Sandy loam	6.9	0.97	8.9	9.1
Speyer 2.4 (II)	Loam	7.2	2.3	25.9	31.4
Speyer 6S (III)	Clay	7.1	1.6	41	25.2
Speyer 2.2 (IV)	Loamy sand	5.4	1.3	6.8	8.55
Am Fischteith (V)	Silt loam	6.2	1.8	20.5	19.5

¹ Table 1 of study, p. 52

Table 2. Details of Tests in Study

Test	Test	Soil-to-solution ratio	Test Concentrations (mg/L)	Tested soils	Adsorption time (hours)
Preliminary	Preliminary	1:1 1:5 1:25	0.188	Sandy loam Loam	2, 5, 24, 29
Screening	Screening Test I	1:25	0.5	All	2, 5, 24, 29
	Screening Test II	1:25	0.5	Sandy loam Clay Loamy sand	2, 5, 24, 28
	Screening Test III	1:50	0.5	All	2, 5, 24, 72
Advanced Adsorption/Desorption	Concentration A-E	1:50 1:50	0.0022-0.2038	All	24

II. RESULTS AND DISCUSSION

The screening tests were used to determine the procedure for conducting the Advanced Test. Screening Test 1 demonstrated the need to sterilize the soil:water systems. Screening Test II established a shaking time of 24 hours for adsorption, and Screening Test III established a shaking time of 24 hours for desorption.

Based on the screening tests, 24 hours was used in the advanced test with a soil:solution ratio of 50:1 in sterilized systems. In the advanced test, the percent sorption of zinc pyrethrin in the five soils from Germany ranged from 12-53 %. The Freundlich Kads values ranged from 28-187 ml/g and the Koc values ranged from 2,088-8,293 ml/g. The fit of the Freundlich equation was good with r^2 values of 0.96-0.99 (Table 1 below). Sorption was most closely related to the organic carbon content of the soils ($r^2=0.85$) and the cation exchange capacity ($r^2=0.69$), which represents the combination of clay, organic carbon content and pH (Table 3 of DER). Based on the log Koc values of 3-4 in tested soils and the FAO soil mobility classification system (FAO)⁹, parent zinc pyrethrin is slightly mobile in soil.

Table 3. Sorption in Five (5) Representative Soils.

Soil (Soil #)	Texture	Percent sorbed	K_f^1 (ml/g)	$K_{foc}^{2,3,4}$ (ml/g)	$1/n^2$	Goodness of fit (r -squared) ²
Speyer 2.3 (I)	Sandy loam	53	34	3,530	0.83	0.966
Speyer 2.4 (II)	Loam	32	187	8,293	1.31	0.987
Speyer 6S (III)	Clay	39	56	3,489	0.81	0.955
Speyer 2.2 (IV)	Loamy sand	12	28	2,088	1.05	0.994
Am Fischteith (V)	Silt loam	36	102	5,620	0.91	0.977

¹ Freundlich adsorption coefficient, K_f , L/kg

² Table 1 of study, p. 52

³ Table in study, p. 49

⁴ $(K_f * 100) / \% \text{ OC}$

III. STUDY DEFICIENCIES AND REVIEWER'S COMMENTS

None.

IV. REFERENCES

Food and Agriculture Organization of the United Nations. APPENDIX 2: Parameters of pesticides that influence processes in the soil.

<http://www.fao.org/docrep/003/x2570e/x2570e06.htm>

Organisation for Economic Cooperation and Development. 1998. OECD Principles of Good Laboratory Practice. ENV/MC/CHEM (98)17, Environmental Directorate, Paris.

OECD (2000), Test No. 106: Adsorption -- Desorption Using a Batch Equilibrium Method,

⁹ <http://www.fao.org/docrep/003/X2570E/X2570E06.htm>

OECD Publishing, Paris. DOI: <http://dx.doi.org/10.1787/9789264069602-en> , <http://www.oecd-ilibrary.org/docserver/download/9710601e.pdf?expires=1487866409&id=id&accname=guest&checksum=34F06A24E18581A81A77AA59D3496137>

U.S. EPA, Fate, Transport, and Transformation Test Guidelines. 2008 OPPTS 835.1230. Adsorption/ Desorption (Batch Equilibrium).

Data Evaluation Record 5


RESPIRATION INHIBITION OF Zinc Pyrithione in ACTIVATED SLUDGE


Report: Fiebig, S. April 8, 2015. Zinc pyrithione: Respiration Inhibition Test with Activated Sludge. Dr. Noeck Project-No. 130429TU, Study-No. BBR15408. Unpublished study Dr. Noeck Laboratories, Germany, and Submitted by THOR GBMH.

Document No.: MRID 49788847

Guideline: OCSPP 850.6800/OCSPP 850.3300/OECD 209

PC Code: 088002

From: James Breithaupt, Agronomist 
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

Peer Review: Diana Hsieh, Biologist 
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

EXECUTIVE SUMMARY

The respiration inhibition test with zinc pyrithione in activated sludge is classified as acceptable for zinc pyrithione and satisfies the OCSPP 850.6800/850.3300/OECD 209 data requirement for ACTICIDE® ZP 100-F. The IC₅₀ value for the reference substance, copper sulfate pentahydrate, was 90 mg/L (between 53 and 155 mg/l) and the IC₅₀ value for ACTICIDE® ZP 100-F (test substance) was determined to be 2.82 mg/L with a 95th confidence interval of 2.69-2.96 mg/l.

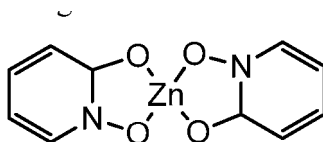
I. MATERIALS AND METHODS

Guideline followed: 850.6800/850.3300/OECD 209

GLP Compliance: This study was conducted in compliance with Directive 2004/10/EC of the European Parliament and of the Council (February 11, 2004), OECD Principles of Good Laboratory Practice ENV/MC/CHEM (99), and German Chemicals Law (August 28, 2013, BGBl. IS. 3498)

A. Materials:

1. Test Material:	ACTICIDE® ZP 100-F , Zinc pyriithione (bis(2-pyridylthio)zinc 1,1'-dioxide)
CAS and IUPAC Name:	Bis(2-pyridylthio)zinc 1,1'-dioxide
CAS Number:	13463-41-7
Molecular formula:	C ₁₀ H ₈ N ₂ O ₂ S ₂ Zn
Molecular weight:	317.7 g/mol
Description:	Solid
Batch Number:	RP-270521-1608 (expiration date August 2016)
Purity:	ACTICIDE® ZP 100-F, 97.55 %;
Storage conditions:	Ambient conditions, protected from light.
SMILES String:	<chem>c2n(cccc2)=O)S[Zn]Sc1n(cccc1)=O</chem>
Chemical Structure:	



Non-adapted activated sludge was collected from the aeration tank at D31135 Hildesheim, Germany because the sludge receives minimal amounts of industrial waste. The sludge was sieved using a 2 mm screen and allowed to settle for approximately 30 minutes. After sieving, the sludge was washed twice with chlorine free tap water and adjusted to a nominal dry sludge concentration of 3.0 g/L \pm 10 %. The observed dry sludge concentration was 2.82 g/L, which was diluted to 1.41 g/L in the test vessels.

Because of the limited solubility of zinc pyriithione (15 mg/L¹⁰), both negative controls and solvent controls were prepared for the test using DMSO as a co-solvent. There were four preliminary range-finding studies to determine the approximate IC₅₀ value and to deal with solubilization issues. The fourth preliminary study used DMSO as a co-solvent and test solutions of 1, 3.2, and 10 mg/L of Zn pyriithione (Table 4 of study).

For the test substance, test concentrations of 0.3125, 0.625, 1.25, 2.5, 5, and 10 mg/l were prepared. In addition to the test substance, aqueous solutions containing 58, 100, and 180 mg/l of copper sulfate pentahydrate were made to determine the IC₅₀ of the reference substance. All of these solutions were made to a volume of 500 ml, and six replicates were used for controls while five replicates were used for the reference and test substance treatments.

Controls (negative and solvent controls), reference, and treatment test mixtures were incubated at 18.0-22.2 °C (average of 20.4 °C) and aerated for three hours at a rate sufficient to provide

¹⁰ This study, MRID 49788847

aerobic conditions and maintain solids in suspension. The mixtures were prepared and aerated in 500 mL Erlenmeyer flasks and then transferred to fill 500 mL biochemical oxygen demand (BOD) bottles to conduct dissolved oxygen (DO) measurements. Test mixtures were identified by project number, test substance identification, test concentration, and bottle number. All mixtures were aerated for three hours.

II. RESULTS AND DISCUSSION

The temperatures throughout the study were 18–22 °C. The initial total suspended solids (TSS) concentration of the activated sludge after removal of the supernatant layer measured 2,820 mg/L. The measured TSS concentration of the sludge was 1,410 mg/l and the measured pH on the day of testing was 7.3-7.4.

The respiration rates observed in the negative control were 24.2-30.8 mg O₂/L/hr (average of 27 mg O₂/L/hr, 6 replicates), and for the solvent control were 25.2-28.4 mg O₂/L/hr (average of 26.6 replicates). The difference (CV, %) within the negative control was 10 %, and for the solvent control, was 5 % (<15 %). The EC₅₀ value for the reference substance was 90 mg/L (between 53 and 155 mg/l).

The observed percent inhibitions for ACTICIDE® ZP 100-F ranged from 1 to 88 %. The IC₅₀ value for the reference substance, copper sulfate pentahydrate, was 90 mg/L (between 53 and 155 mg/l) and the IC₅₀ value for ACTICIDE® ZP 100-F (test substance) was determined to be 2.82 mg/L with a 95th confidence interval of 2.69-2.96 mg/l.

III. STUDY DEFICIENCIES AND REVIEWER’S COMMENTS

None.

IV. REFERENCES

Organisation for Economic Cooperation and Development: OECD 209. July 22, 2010. OECD Guidelines for the Testing of Chemicals: Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation).

U.S. EPA., Ecological Effects Test Guidelines, OPPTS 850.6800, Modified Activated Sludge Respiration Inhibition Test for Sparingly Soluble Chemicals, “Public Draft.” EPA 712-C-96-168.

U.S. Environmental Protection Agency. 2012. Series 850- Ecological Effects Test Guidelines. OCSPP Number 850.3300: Modified Activated Sludge, Respiration Inhibition Test.

Data Evaluation Record 6

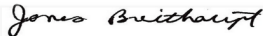
BIODEGRADABILITY OF ZINC PYRITHIONE (CAS 13463-41-7) IN THE POROUS POT WWTP SIMULATION TEST (OCSPP 835.3220)


Report: Schaefer, E.; Ponizovsky, A.; Ashchyan, T. (2015) Zn Pyrithione: Porous Pot Test Method for Assessing the Biodegradability of the Test Substance During Wastewater Treatment Simulation. Project Number: 578/052814/OECD/303A/SUB578, 578E/102. Unpublished study prepared by Wildlife International Ltd. 133p.

Document No.: MRID 49788844

Guideline: OCSPP 835.3220

PC Code: 088002

From: James Breithaupt, Agronomist 
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

Peer Review: Diana Hsieh, Biologist 
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

EXECUTIVE SUMMARY

This study is classified as acceptable for zinc pyrithione and satisfies the OCSPP 835.3220 data requirement for ACTICIDE® ZP 100-F. Zinc pyrithione is expected to degrade rapidly in water or during wastewater treatment because 94-99 % was removed during the porous pot test at 0.2 and 0.5 mg/L. The primary degradation products were pyrithione sulfonic acid and pyridine sulfonic acid. For the 0.2 mg/L treatment rate, the routes of dissipation were, in descending order, non-extractable sorption to sludge (20 %), mineralization (13 %), and release in effluent (1.2 %). For the 0.5 mg/L treatment rate, the routes of dissipation were, in descending order, non-extractable sorption to sludge (33 %), release in effluent (25 %), and mineralization (9 %). At 0.2 mg/L, zinc pyrithione was toxic to WWTP organisms in the acclimation phase of the study based on the increased dissolved organic carbon (DOC content), but was not determined to be toxic in the steady-state phase. However, at 0.5 mg/L, zinc pyrithione was observed to be toxic to WWTP microorganisms in both the acclimation and steady-state phases. Even so, the Chemical Oxygen Demand (COD) was not affected at either concentration.

I. MATERIALS AND METHODS

Guideline followed: OCSPP 835.3220

GLP Compliance: This study was conducted in compliance with EPA GLP regulations 40 CFR Part 792, 1989 and OECD Principles of Good Laboratory Practice ENV/MC/CHEM (98).

A. Materials:

1. Test Material: ACTICIDE® ZP 100-F, Zinc pyriothione (bis(2-pyridylthio)zinc 1,1'-dioxide)

IUPAC Name: Bis(2-pyridylthio)zinc 1,1'-dioxide

CAS Name: Bis(2-pyridylthio)zinc 1,1'-dioxide

CAS Number: 13463-41-7

Molecular formula: C₁₀H₈N₂O₂S₂Zn

Molecular weight: 317.7

Description: Solid

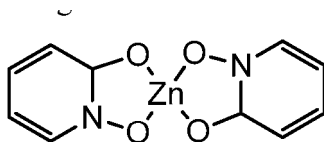
Batch Number: 4794NGP034-1

Purity: ACTICIDE® ZP 100-F, Radiopurity, 99.5 %, HPLC/UV purity, 99.6 %, Specific activity 43.27 mCi/mmol (135.6 µCi/mg);

Storage conditions: Ambient conditions.

SMILES String: c2(n(cccc2)=O)S[Zn]Sc1n(cccc1)=O

Chemical Structure:



Test Inoculum (Activated Sludge)

Secondary activated sludge was obtained from the wastewater treatment plant at Easton, Maryland on July 18, 2014 which primarily treats domestic wastewater. The sludge was sieved through a 2-mm screen and the total suspended solids (TSS) was adjusted to 2,500 mg/L before being placed into each porous pot reactor. Figure 1 of the study contains a diagram of the study apparatus (See Appendix A).

Nutrition Source (Domestic Sludge)

Domestic sludge from the Cambridge Wastewater Treatment Facility, Cambridge, MD because the sewage from this facility was not known to contain any contaminants that could interfere with the study. This sludge was sieved using a 2-mm mesh screen prior to use. The chemical oxygen demand (COD) associated with this sewage was determined prior to the study and was diluted to <400 mg/L using tapwater. The TSS was determined in the initial and diluted sewage, followed by continuous mixing and storage under refrigeration.

Porous Pot Reactors and Stages of Study

There were three porous pot reactors used in the test, and each bioreactor was a glass vessel with a porous polyethylene membrane cylinder inside (Figure 1 of study, See Appendix A). The tubes for pumping influent sewage, dosing solution, and providing air for aeration of the mixed liquor were sealed in the openings of the lid for the reactor.

The stabilization test with a duration of three weeks was conducted to allow the activated sludge to adapt to laboratory conditions in the absence of the test substance. For the stabilization test with a duration of two weeks, approximately 1.13 L of the activated sludge mixed liquor containing TSS concentrations of 2,500 mg/L was placed in the membrane within the three reactors. Mixed liquor is a mixture of raw or settled wastewater (domestic sludge) and activated sludge contained in an aeration basin in the activated sludge process. In a mixed liquor, mixed liquor suspended solids (MLSS) is the concentration of suspended solids in mixed liquor, usually expressed in mg/L¹¹. During this phase, dissolved organic carbon (DOC), chemical oxygen demand (COD), pH, and dissolved oxygen (DO) were measured.

The acclimation test with a duration of 12 days was conducted to allow the stabilized test systems to adjust to the presence of the test substance. The zinc pyrithione concentrations were 0 mg/L (control) in Reactor 1, 0.2 mg/L in Reactor 2, and 0.5 mg/L in Reactor 3. The steady test period with a duration of 21 days was conducted to determine the biodegradability and sorption of the test substance at the zinc pyrithione concentrations used in the acclimation study. All of these study stages were conducted at 20 ± 1 °C.

For the inoculum and silica gel control, 10 bottles contained silica gel, and inoculum were prepared. For the definitive test, 10 bottles containing inoculum, silica gel, and test substance were prepared. Also, controls were prepared for inoculum only (6 bottles) and sodium acetate and inoculum (6 bottles). Sodium acetate is a readily biodegradable reference compound whose degradation results are used to interpret the results for the test substance. The concentrations of the test substance and sodium acetate in the bottles were 2.0 and 6.7 mg/L, respectively. Two g of silica gel were added to the bottles and each of the prepared solutions was dispensed into the respective group of BOD bottles so that all bottles were completely filled without air bubbles. The zero time bottles were immediately analyzed for dissolved oxygen using an oxygen electrode. The remaining bottles were closed and incubated in darkness and duplicate bottles were withdrawn at day 7, 14, 21, and 28.

¹¹ Water online, 2017, <https://www.wateronline.com/doc/mixed-liquor-suspended-solids-in-wastewater-0002>

The dissolved oxygen concentrations were determined electrochemically using an oxygen electrode and the pH and temperature were measured.

II. RESULTS AND DISCUSSION

Stabilization Period

During the stabilization period, the sludge retention time (SRT) was 10 days in all reactors and the hydraulic retention time (HRT) was 6.9 hours (Table 2 of study). The sludge retention time is the average time that the activated sludge solids are present in the system (Lenntech), and is the ratio of the MLSS in the aeration tank to the TSS of the influent entering the aeration tank¹², The SRT of 10 days is in the range of 3-30 days (State of Mississippi)¹³. The HRT, or water treatment time, of 6.9 hours is consistent with the range of treatment times (6-8 hours) in a full-scale system (Boethling)¹⁴. In the stabilization period, DOC removal in all reactors was stabilized at the level of 38.7% to 41.0% (Table 3 of study) and COD removal was 95% to 97% (Table 4 of study). In the stabilization period, the pH in the mixed liquor ranged from 7.4-7.8 and the DO ranged from 3.8-4.4 \pm 2.2 mg/L, indicating that relatively consistent conditions were present (Table 2 of study).

Acclimation Period

Dissolved Organic Carbon (DOC) in Effluent

Reactor 1 (control reactor) received no zinc pyrethione, and Reactor 2 received 0.2 mg/L of zinc pyrethione. In the control reactor, the DOC was similar to that of the stabilization period with 40 % reduction of effluent DOC. Reactor 2 demonstrated an increase of 259 % in effluent DOC because of microbial inhibition by the end of the acclimation period. Reactor 3, treated with 0.5 mg/L zinc pyrethione, demonstrated an increase of 167 % in effluent DOC by the end of the acclimation period because of microbial inhibition (Table 3 of study).

Chemical Oxygen Demand (COD) in Effluent

During the acclimation phase, the chemical oxygen demand (COD) was only slightly affected by the concentration of zinc pyrethione. In the control reactor, 87 % of COD was removed while 80 % was removed in Reactor 2 (0.2 mg/l) and Reactor 3 (0.5 mg/L).

¹² Solids retention time (SRT)=MLSS in aeration tank/basin/ TSS Entering Aeration Tank

¹³ State of Mississippi,

[http://www.deq.state.ms.us/MDEQ.nsf/8f3cca80758d46df86256c080014bab8/557702f42378ab3b862573220073b407/\\$FILE/QP7498-DEQ-Chapter%205%20corrections_pg%2054.pdf](http://www.deq.state.ms.us/MDEQ.nsf/8f3cca80758d46df86256c080014bab8/557702f42378ab3b862573220073b407/$FILE/QP7498-DEQ-Chapter%205%20corrections_pg%2054.pdf)

¹⁴ November 22, 2013 e-mail from Bob Boethling of EPA to Pat Jennings of EPA

Steady State Period

Dissolved Organic Carbon (DOC) in Effluent

In the control reactor (Reactor 1), the percent removal of DOC in the effluent was 21-69 % (average of 57 %) with 9 of 10 removal values between 54 and 69 %. In Reactor 2 treated using 0.2 mg/L zinc pyrithione, the percent elimination values ranged from 23-68 % (mean of 54 %) with one outlier value of -55 %. Based on the results in Reactor 2, zinc pyrithione at 0.2 mg/L was not toxic to the WWTP microorganisms after the acclimation period but may be initially toxic to unacclimated microorganisms based on the increase in DOC in the effluent. In Reactor 3, treated using 0.5 mg/L zinc pyrithione, the percent elimination of DOC from effluent was initially -148 %, decreased to -292 %, and then increased to -11 %, indicating that the toxicity to WWTP organisms was preventing degradation. As a result, the effluent DOC concentrations in Reactor 3 were higher than the influent (Table 3 of study).

Chemical Oxygen Demand (COD) in Effluent

In contrast to the DOC, the COD was relatively unaffected by the presence of zinc pyrithione at concentrations of up to 0.5 mg/L. (Table 4 of study). The COD reduction was 88 % in the control, 92 % at 0.2 mg/L, and 82 % at 0.5 mg/L.

Degradation of Parent Compound and Formation of Degradation Products in the Steady-State Test

Reactor 2 (0.2 mg/L zinc pyrithione)

In this study, zinc pyrithione at concentrations of 0.2 and 0.5 mg/L formed numerous compounds based on Chromatograms 8-23 in the study. In the effluent from Reactor 2 (1.2 % of applied radioactivity), 75-100 % (average of 94.2 %) of parent zinc pyrithione degraded into other residues (Table 11 of study). Mineralization ranged from 7-19 % (average of 14 %) in the acclimation phase and 6-26 % (average of 13 %) in the steady-state phase (Table 8 of study). In effluent from the steady-state phase, parent compound ranged from 0-25 % of radioactivity (average of 5.9 %) with most values being <5.5 %. There were two significant (>10 % of applied) degradation products and one non-significant degradation product formed (Table 10 of study), but these were not identified because the test guideline does not require identification of degradation products.

Reactor 3 (0.5 mg/L zinc pyrithione)

In the effluent from Reactor 3, 9-49 % of applied radioactivity (average of 25 %) was present in effluent in the steady state period (Table 19). Mineralization ranged from 7-25 % (average of 15 %) in the steady-state phase (Table 20 of study). Virtually all zinc pyrithione (91-100%, average of 98 %) degraded into other residues (Table 21 of study). In effluent from the steady-state phase, parent compound ranged from 0-9 % of radioactivity (average of 2.3 %) with most values being <3 %. There was one significant (>10 % of applied) degradation product and there were three non-significant degradation product formed (Table 21 of study).

Partitioning of Radioactive Residues

Table 12 of the study provides information on the partitioning of zinc pyriithione residues for 0.2 mg/L, and Table 22 of the study provides the same information for 0.5 mg/L zinc pyriithione. The results of these study tables are summarized in Table 1 of the DER below.

Table 1. Partitioning of Total Radioactivity Residues in Mixed Liquor in Mixed Liquor in Steady State Period (%)

Compartment	0.2 mg/L Range (average)	0.5 mg/L Range (average)
Supernatant (liquid component)	0.8-3.3 (1.2) ¹	14-49 (25) ³
Extractable from solids	1.0-3.4 (2.5) ¹	2.3-13 (7.5) ³
Non-extractable from solids	13-32 (20) ¹	21-57 (33) ³
CO ₂ (effluent plus volatility traps)	6-26 (13) ²	0.5-16 (9) ⁴

¹ Table 12 of study

² Table 9 of study

³ Table 22 of study

⁴ Table 20 of study

In this study, zinc pyriithione at concentrations of 0.2 and 0.5 mg/L formed numerous compounds based on Chromatograms 8-23 of the study. In Reactor 2 (Table 13 of study) and Reactor 3 (Table 21 of study), numerous degradation products were formed.

III. STUDY DEFICIENCIES AND REVIEWER'S COMMENTS

Numerous significant (>10 % of applied) degradation products were formed in this study but were not identified. However, the study is not invalidated because (1) the 835.3220 test guideline does not require degradate identification and (2) the lack of zinc pyriithione persistence was consistent with other environmental fate studies.

IV. REFERENCES

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MRID 49788848. Adam, D. (2015) Zn-[Carbon 14] Pyriithione: Hydrolysis at Three Different pH Values: Final Report. Project Number: 20130082. Unpublished study prepared by Innovative

Environmental Services (IES) Ltd. 136p.

MRID 49788849. Adam, D. (2015) Zn-[Carbon 14] Pyrithione: Aqueous Photolysis in Buffer Solution and Natural Water: Final Report. Project Number: 20130084. Unpublished study prepared by Innovative Environmental Services (IES) Ltd. 100p.

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State of Mississippi, Department of Environmental Quality.

[http://www.deq.state.ms.us/MDEQ.nsf/8f3cca80758d46df86256c080014bab8/557702f42378ab3b862573220073b407/\\$FILE/QP7498-DEQ-Chapter%205%20corrections_pg%2054.pdf](http://www.deq.state.ms.us/MDEQ.nsf/8f3cca80758d46df86256c080014bab8/557702f42378ab3b862573220073b407/$FILE/QP7498-DEQ-Chapter%205%20corrections_pg%2054.pdf)

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<https://www.wateronline.com/doc/mixed-liquor-suspended-solids-in-wastewater-0002>

Appendix A. Porous Pot Reactor from Figure 1 of MRID 49788844.

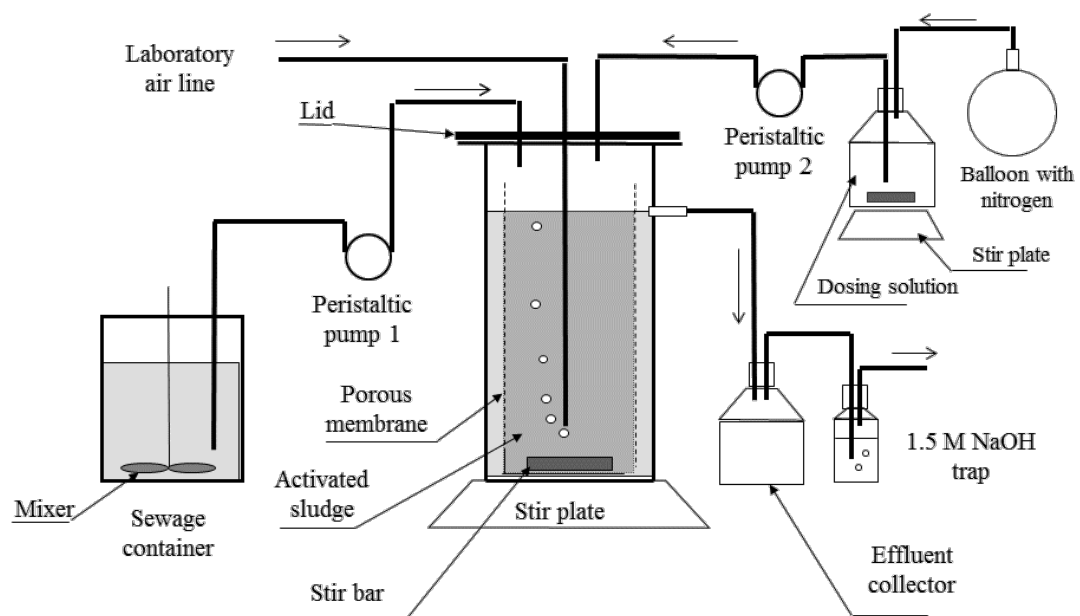


Figure 1. Schematic diagram of porous pot reactor test system

Data Evaluation Record 7


ANALYTICAL METHOD VALIDATION OF ZINC PYRITHIONE IN WATER


Citation: Buttler, O. (2015) Zinc Pyrithione: Residue Analytical Method for the Determination in Water. Project Number: CRA15408, 130528TS. Unpublished study prepared by Dr. U. Noack-Laboratorien. 60p.

Document No.: MRID 49788850

Guideline: OCSPP 850.6100

PC Code: 088002

From: James Breithaupt, Agronomist 
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

Peer Review: Diana Hsieh, Biologist 
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

EXECUTIVE SUMMARY

The analytical method validation study (MRID 49788850) is classified as acceptable and satisfies the 850.6100 data requirement for water for zinc pyrithione formulated as ACTICIDE® ZP 100-F. In this study, recoveries of zinc pyrithione in drinking water, surface water, and ground water were within the 70-120 % recovery required by the test guideline, and the relative standard deviations (RSD or CV, %) were less than the 20 % required by the test guideline.

I. MATERIALS AND METHODS

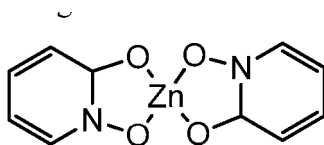
Guideline followed: The Buttler (2015) study is acceptable and satisfies the 835.1230 data requirement for zinc pyrithione.

GLP Compliance: This study was conducted in compliance with OECD Principles of Good Laboratory Practice (April, 1999), Directive 2004/10/EC of the European Parliament and of the Council of 11th February 2004, Part two, and OECD principles of Good Laboratory Practice, adopted by Council on 26th November 1997; Environment Directorate, Organisation for Economic Cooperation and Development, April 1999.

A. Materials:

1. Test Material: ACTICIDE® ZP 100-F, Zinc pyriithione (bis(2-pyridylthio)zinc 1,1'-dioxide)

CAS and IUPAC Name: Bis(2-pyridylthio)zinc 1,1'-dioxide
CAS Number: 13463-41-7
Molecular formula: C₁₀H₈N₂O₂S₂Zn
Molecular weight: 317.7 g/mol
Description: Solid
Batch Number: 4794NGP036-2 (no stated expiration date)
Purity: ACTICIDE® ZP 100-F, 97.55 %;
Storage conditions: Ambient conditions.
SMILES String: c2(n(cccc2)=O)S[Zn]Sc1n(cccc1)=O
Chemical Structure:



Test Procedures and Analytical Methods

Analytical method validation was conducted using drinking water, surface water, and ground water from Germany. Table 1 of this DER contains the chemical and physical properties of the different water sources.

Table 1. Chemical and physical properties of water sources

Property	Drinking water ¹	Innerate River Surface water ²	Ground water ³
Sampling date	7-18-2014	6-23-2014	7-1-2014
pH	8.22	8.21	7.29
Conductivity (µS/cm)	165.2	972	1355
Dissolved oxygen (%)	111.9	109.9	89.1
Dissolved organic carbon (DOC, mg C/L)	4.34	3.54	14.14
Ammonium nitrogen (mg N/L)	<0.04	0.08	<0.04
Nitrate nitrogen (mg N/L)	1.1	1.9	<0.3
Storage condition	Room temperature	15.2 ± 2 °C	Room temperature

¹ Table 3 of study

² Table 4 of study

³ Table 5 of study

A stock solution of 100 mg 1-Hydroxypyridine-2-thione zinc salt/L was prepared in dimethylsulfoxide and diluted to 10 mg/l and further to 100 µg/L with HPLC water: acetonitrile (60:40). This solution was further diluted with water:acetonitrile (60:40) solution to 8 concentrations and placed into 1.5 ml brown-glass vials. One (1.0) ml of each standard was derivatized with 50 µL NBD-F¹⁵ (20 g/L in acetonitrile) for 2 hours at approx. 70 °C and used for calibration.

Spiking solutions of 0.1 µg/L (LOQ) and 1 µg/L (10X LOQ) and 10, 100, 1000, 10,000 mg/L zinc pyriithione in DMSO were prepared. Five (5) replicates were prepared for each spiking solution for drinking water, surface water, and ground water and two (2) control samples with no zinc pyriithione were prepared for each water source. In addition, two (2) replicates of each LOQ samples for each water source were used for storage stability testing.

RESULTS AND DISCUSSION

Recoveries of zinc pyriithione in drinking water were 115 % for 0.1 µg/L (LOQ) and 105 % for 1 µg/L (10X LOQ), which is within the acceptable range of 70-120 %. The variability between replicates was 4.3 % for the LOQ and 2.9 % for 10X LOQ, which is less than the 20 % relative standard deviation (RSD), or coefficient of variability (CV) of 20 %.

Recoveries of zinc pyriithione in surface water were 94 % for 0.1 µg/L (LOQ) and 93 % for 1 µg/L (10X LOQ), which is within the acceptable range of 70-120 %. The variability between replicates was 8.6 % for the LOQ and 4.3 % for 10X LOQ, which is less than the 20 % RSD, or CV of 20 %.

Recoveries of zinc pyriithione in ground water 74 % for 0.1 µg/L (LOQ) and 76 % for 1 µg/L (10X LOQ), which is within the acceptable range of 70-120 %. The variability between replicates was 8.1 % for the LOQ and 5.3 % for 10X LOQ, which is less than the 20 % relative standard deviation (RSD), or coefficient of variability (CV) of 20 %.

References:

OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation. EPA 712-C-001. <https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0154-0008>

¹⁵ 4-Fluoro-7-nitrobenzofurazan, CAS No. [29270-56-2](#)

Data Evaluation Record 8


ANALYTICAL METHOD VALIDATION OF ZINC PYRITHIONE IN SOIL


Citation: Buttler, O. (2015) Zinc Pyrethrin: Residue Analytical Method for the Determination in Soil. Project Number: 150505TS, CRA15408. Unpublished study prepared by Dr. U. Noack-Laboratorien. 53p.

Document No.: MRID 49788851

Guideline: OCSPP 850.6100

PC Code: 088002

From: James Breithaupt, Agronomist 
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

Peer Review: Diana Hsieh, Biologist 
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

EXECUTIVE SUMMARY

The analytical method validation study (MRID 49788851) is classified as acceptable and satisfies the 850.6100 data requirement for zinc pyrethrin formulated as ACTICIDE® ZP 100-F. Recoveries of zinc pyrethrin in soil were 36-49 % for 0.015 mg/kg (LOQ) and 39-47 % for 0.15 mg/kg (10X LOQ), which are clearly not in the acceptable range of 70-120 %. However, in the aerobic soil metabolism study (MRID 49788845), zinc pyrethrin degraded with a half-life of <30 minutes in the Lufa 2.3 soil which was common to both the aerobic soil metabolism study and the method validation study. As a result, these low recoveries are expected and the study is not invalidated. In addition, the Relative Standard Deviations were 12.5 % for the LOQ treatment and 7.3 % for the 10X LOQ treatment, both of which are less than the 20 % allowed by the test guideline.

I. MATERIALS AND METHODS

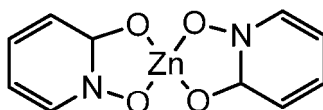
Guideline followed: The Buttler (2015) study is acceptable and satisfies the 835.1230 data requirement for zinc pyrethrin.

GLP Compliance: This study was conducted in compliance with OECD Principles of Good Laboratory Practice (April, 1999), Directive 2004/10/EC of the European Parliament and of the Council of 11th February 2004, Part two, and OECD principles of Good Laboratory Practice, adopted by Council on 26th November 1997; Environment Directorate, Organisation for Economic Cooperation and Development, April 1999.

A. Materials:

1. Test Material: ACTICIDE® ZP 100-F, Zinc pyriithione (bis(2-pyridylthio)zinc 1,1'-dioxide)

CAS and IUPAC Name: Bis(2-pyridylthio)zinc 1,1'-dioxide
CAS Number: 13463-41-7
Molecular formula: C₁₀H₈N₂O₂S₂Zn
Molecular weight: 317.7 g/mol
Description: Solid
Batch Number: 4794NGP036-2 (no stated expiration date)
Purity: ACTICIDE® ZP 100-F, 97.55 %;
Storage conditions: Ambient conditions.
SMILES String: c2(n(cccc2)=O)S[Zn]Sc1n(cccc1)=O
Chemical Structure:



Test Procedures and Analytical Methods

Analytical method validation was conducted using the LUFA 2.3 soil from Germany (non-sterilized) with a sandy loam texture. This soil had a pH of 5.8 and an organic carbon content of 0.67 %.

A stock solution of 100 mg 1-Hydroxypyridine-2-thione zinc salt/L was prepared in dimethylsulfoxide and diluted to 10 mg/l and further to 100 µg/L with HPLC water: acetonitrile (60:40). This solution was further diluted with water:acetonitrile (60:40) solution to 8 concentrations and placed into 1.5 ml brown-glass vials. One (1.0) ml of each standard was derivatized with 50 µL NBD-F¹⁶ (20 g/L in acetonitrile) for 2 hours at approx. 70 °C and used for calibration.

Spiking solutions of 1.5 mg/L and 15 mg/L were prepared. Five (5) replicates were prepared for each spiking solution for the soil (5 g) treated at the limit of quantitation (LOQ) of 0.015 mg/kg. In addition, five (5) replicates were prepared for each spiking solution for the soil (5 g) treated at 10 times (10X) the (LOQ) of 0.15 mg/kg. Two negative control samples of 5 g soil (untreated) were also prepared.

¹⁶ 4-Fluoro-7-nitrobenzofurazan, CAS No. [29270-56-2](#)

The soils were extracted using dichloromethane:methanol (97:3) and the extracts were analyzed using HPLC connected sequentially to two MS detectors.

RESULTS AND DISCUSSION

Recoveries of zinc pyrethrin in soil were 36-49 % for 0.015 mg/kg (LOQ) and 39-47 % for 0.15 mg/kg (10X LOQ), which are not in the acceptable range of 70-120 %. However, in the aerobic soil metabolism study (MRID 49788845), zinc pyrethrin degraded with a half-life of <30 minutes in the Lufa 2.3 soil which was common to both the aerobic soil metabolism study and the method validation study. As a result, these low recoveries are expected and the study is not invalidated. In addition, the Relative Standard Deviations were 12.5 % for the LOQ treatment and 7.3 % for the 10X LOQ treatment, both of which are less than the 20 % allowed by the test guideline.

References:

MRID 49788845. Volkel, W. (2015) Zn-[Carbon 14]Pyrethrin - Route and Rate of Degradation in Four Soils Incubated Under Aerobic Conditions: Final Report. Project Number: 20130088. Unpublished study prepared by Innovative Environmental Services (IES) Ltd. 145p.

OCSP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation. EPA 712-C-001. <https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0154-0008>

Data Evaluation Record 9


NITROGEN AND CARBON TRANSFORMATION TEST FOR ZINC PYRITHIONE


Report: Winkelmann, G. (2014) Zinc Pyrithione: Soil Micro-Organisms: Nitrogen Transformation Test. Project Number: 130429TU, TBN15408. Unpublished study prepared by Dr. U. Noack-Laboratorien. 54p. (MRID 49788842)

Winkelmann, G. (2015) Zinc Pyrithione: Soil Micro-Organisms: Carbon Transformation Test. Project Number: 130429TU, TBN15408. Unpublished study prepared by Dr. U. Noack-Laboratorien. 54p. (MRID 49788843)

Guideline: OECD 216 (nitrogen transformation) and OECD 217 (carbon transformation)

PC Code: 088002

From: James Breithaupt, Agronomist 
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

Peer Review: Diana Hsieh, Biologist 
Risk Assessment and Science Support Branch (RASSB)
Antimicrobial Division (7510P)

EXECUTIVE SUMMARY

These studies (MRIDs 49788842 and 49788843) are both classified as acceptable but are not required to satisfy 158W data requirements for zinc pyrithione. Zinc pyrithione is not expected to inhibit either nitrification or carbon transformation in soil based because the 50 % inhibition value for nitrification was 222 mg/kg (equivalent to lbs/A) and inhibition of carbon transformation was not observed following acclimation (7-28 days).

I. MATERIALS AND METHODS

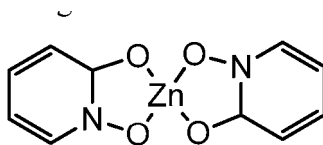
Guideline followed: The Winkelmann (2015) study is acceptable and satisfies the OECD 216 data requirement for zinc pyrithione.

GLP Compliance: This study was conducted in compliance with OECD Principles of Good Laboratory Practice (April, 1999), Directive 2004/10/EC of the European Parliament and of the Council of 11th February 2004, Part two, and OECD principles of Good Laboratory Practice, adopted by Council on 26th November 1997; Environment Directorate, Organisation for Economic Cooperation and Development, April 1999.

A. Materials:

1. Test Material: ACTICIDE® ZP 100-F, Zinc pyriithione (bis(2-pyridylthio)zinc 1,1'-dioxide)

CAS and IUPAC Name: Bis(2-pyridylthio)zinc 1,1'-dioxide
CAS Number: 13463-41-7
Molecular formula: C₁₀H₈N₂O₂S₂Zn
Molecular weight: 317.7 g/mol
Description: Solid
Batch Number: RP-270521-1608 (no stated expiration date)
Purity: ACTICIDE® ZP 100-F, 97.55 %
Storage conditions: Ambient conditions.
SMILES String: c2(n(cccc2)=O)S[Zn]Sc1n(cccc1)=O
Chemical Structure:



Nitrogen Transformation

Freshly-collected Lufa Speyer D67346 (Lufa 2.3) sandy loam soil (1.1 % organic carbon, CEC of 54 mmol/kg, pH of 6.8). was sampled to a depth of 20 cm (8 inches) and sieved to a particle size of up to 2 mm (Table 3 of study, see Appendix A). The soil was then adjusted to 50 % of its maximum water holding capacity (38.3 % w/w) with demineralized water. The soil was amended using powdered alfalfa (legume, also called Lucerne) and green gas mixture containing a C:N ratio of 12:1 to 16:1 at 5 g Lucerne/kg of soil. The treated soil was stored for 92 days in darkness at 6 °C. After storage, the soil was pre-incubated at room temperature (13-22 °C) for 19 days for adaptation to the test system.

A control soil (400 g) was amended using 1 % (w/w) quartz sand. In addition, zinc pyriithione was applied at 2.56, 6.4, 16, 40, 100, and 250 mg/Kg dry soil weight which is equivalent to lbs/A. A positive control using the toxic reference compound, cyanoguanidine¹⁷, was not conducted in this study. However, according to the study, this test is run once per year with 50 and 100 mg/kg dry weight soil and the results were provided in Table 15 of the study.

¹⁷ CAS No. 461-58-5, 64 % nitrogen (w/w), see Appendix A for structure

Carbon Transformation

A freshly-collected Lufa Speyer D67346 (Lufa 2.3) sandy loam soil (1.1 % organic carbon, CEC of 54 mmol/kg, pH of 6.8). was sampled to a depth of 20 cm (8 inches) and sieved to a particle size of up to 2 mm (Table 3 of study). The soil was then adjusted to 50 % of its maximum water holding capacity (38.3 % w/w) with demineralized water. The soil was amended using glucose at 4,000 mg/kg to provide immediate maximum respiration. The treated soil was stored for 24 hours in darkness at 20 °C. After storage, the soil was pre-incubated at room temperature (13-22 °C) for 19 days for adaptation to the test system.

A control soil (2,000 g) was amended using 1 % (w/w) quartz sand. In addition, zinc pyrithione was applied at 2.56, 6.4, 16, 40, 100, and 250 mg/Kg dry soil weight which is equivalent to lbs/A. A positive control using the toxic reference compound, Dinoterb¹⁸ ((2-(Methyl-2-propenyl)-4,6-dinitrophenol), was not conducted in this study. However, according to the study, this test is run once/year added at 18.75 mg/kg dry weight soil and the results were provided in Table 13 of the study.

II. RESULTS AND DISCUSSION

Nitrogen Transformation

The registrant conducted a non-GLP screening test (Table 4 of study, see Appendix A) using concentrations of 1, 10, 100, and 1,000 mg zinc pyrithione/kg dry soil at 0, 7, 14, and 28 days. There was an acclimation to the presence of zinc pyrithione by the soil bacteria based on the stimulation observed at 1 mg/kg (2-34 %), 10 mg/kg (9-63 %), and 100 mg/kg (41-59 %). However, at 1,000 mg/kg, the inhibition ranged from 75-96 %. Because of the variability of nitrification rates, a “significant” increase or decrease is 25 % based on the test guideline.

In the GLP test, zinc pyrithione also exhibited both stimulation and inhibition. At 7 days, stimulation increased to 72 % at up to 16 mg/kg, and inhibition increased from 31 % at 40 mg/kg to 100 % at 250 mg/kg. At 14 days, stimulation increased to 91 % at 40 mg/kg, and increased from 30 % at 100 mg/kg to 110 % at 250 mg/kg. At 28 days, stimulation increased to 54 % at 100 mg/kg and inhibition was 65 % at 250 mg/kg (Table 6 of study, see Appendix A).

The positive control test data using cyanoguanidine provided questionable results based on Tables 14 and 15 of the study. Table 14 of the study contains the mean nitrate nitrogen in the reference item test for the control, 50 mg/kg treatment, and 100 mg/kg treatment. Based on Table 14 of the study and Agency calculations, stimulation was present at 0.6 % and inhibition ranged from 8-21 % for 50 mg/kg, and for 100 mg/kg, inhibition increased from 2 % at day zero to 60 % at day 28. Table 15 contains inhibition rates for 7, 14, and 28 days that are apparently unrelated to the data in Table 14, including 14-52 % for 50 mg/kg and 70-100 % for 100 mg/kg.

For a study to be valid, variation of nitrate-N contents between control replicates must be <15 % at 0, 7, 14, and 28 days. In this study, the variability ranged from 5-6 %.

¹⁸ CAS No. 1720-07-1, 50 % carbon (w/w), see Appendix A for structure

Table 1. Nitrification Endpoints for Zinc Pyrithione¹.

Endpoint (mg/kg dry weight)	Day 7 (range)	Day 14 (range)	Day 28 (range)
NOAEC	2.56 (none)	2.56 (none)	16 (none)
LOAEC	6.4 (none)	6.4 (none)	40 (none)
EC ₅₀	47.7 (39.4-56.6)	119 (109-129)	222 (195-250)

¹ Based on Table 7 of study

Carbon Transformation

The registrant conducted a non-GLP screening test (Table 4 of study) using concentrations of 1, 10, 100, and 1,000 mg zinc pyrithione/kg dry soil at 0, 7, 14, and 28 days. Generally speaking, there was an acclimation to the presence of zinc pyrithione by the soil bacteria based on the stimulation observed at all tested concentrations ranging from 9 % to 70 % at 1,000 mg/kg at 28 days. The only significant (>25 %) inhibition observed was 41 % at 100 mg/kg and 55 % inhibition at 1,000 mg/kg at day zero.

In the GLP test, zinc pyrithione also exhibited both stimulation and inhibition. At 0 days, inhibition ranged from 11 % at 2.56 mg/kg and increased to 53 % at 250 mg/kg. However, by Day 7, stimulation ranging from 3 % (2.56 mg/kg) to 60 % (250 mg/kg) occurred. At Day 14, stimulation reached significant amounts of 46-60 % at 100-250 mg/kg. At Day 28, only 20 % inhibition was observed at 100 mg/kg and 60 % stimulation was observed at 250 mg/kg (Table 5 of study).

The positive control test data using Dinoterb indicated that inhibition increased over time. Simulation (19 %) was observed at zero days and inhibition increased to 34 % (>25 %) by 28 days. Based on a >25 % inhibition at Day 28, these results are valid.

For a study to be valid, variation of glucose metabolism between control replicates must be <15 % at 0, 7, 14, and 28 days. In this study, the variability ranged from 11-18 %. However, the study is not invalidated because stimulation of metabolism by zinc pyrithione was observed in the study after adaptation with minimal inhibition being observed. As a result, carbon transformation endpoints (NOAEC, LOAEC, and EC₅₀) were not calculated.

III. STUDY DEFICIENCIES AND REVIEWER'S COMMENTS

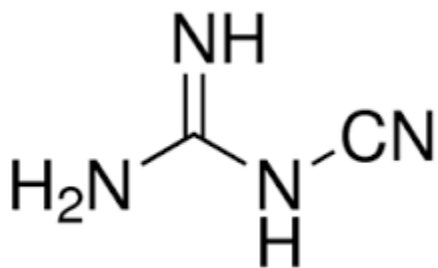
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IV. REFERENCES

Organisation for Economic Cooperation and Development. 1998. OECD Principles of Good Laboratory Practice. ENV/MC/CHEM (98)17, Environmental Directorate, Paris.

OECD Guideline 217 (January 2000): Soil Microorganisms: Carbon Transformation Test

Appendix A. Structures of Toxic Reference Compounds



Cyanoguanidine (2-cyanoguanidine, CAS No. 461-58-5)



Dinoterb (2-(2-Methyl-2-propanyl)-4,6-dinitrophenol, CAS No. 1420-07-1)